RIDA®GENE Sapovirus

REF PG1605

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Sapovirus is a multiplex real-time RT-PCR for the direct, qualitative detection of Sapovirus from human stool samples.[1]

RIDA®GENE Sapovirus real-time RT-PCR is intended for use as an aid in diagnosis of gastroenteritis caused by sapoviruses.

2. Summary and Explanation of the test

Sapoviruses, also called Sapporo Virus belong to the family of *Caliciviridae* and are classified as Sapporo-like viruses.[2] Together with the Norowalk-like viruses (Norovirus) they are the major causative agents of gastroenteritis worldwide. The name Sapovirus originated from the place of its first discovery, Sapporo, Japan in 1977 in an orphanage for small children. Even though highest incidence is described for children under the age of five, other studies also show Sapovirus outbreaks in adults.[3] Clinical symptoms are similar to those of Norovirus infections including diarrhea, vomiting and fever. However self-limited Sapovirus infections lead to much milder gastroenteritis compared to Norovirus-induced gastroenteritis.
After its first discovery in Japan, further Sapovirus outbreaks were described in countries worldwide such as the USA, Canada, Africa and South Africa.[4,5,6] There are at least 5 genogroups (GI – GV) described with GI, GII, GIV and GV being known to infect humans. Until today, little epidemiologic studies were conducted and Sapovirus was rarely detected due to lack of sensitive detection methods. Hence, real-time RT-PCR displays a major advantage in detection of gastroenteritis-causing Sapovirus infections.

3. Test principle

The RIDA®GENE Sapovirus multiplex real-time RT-PCR is a molecular diagnostic test for the direct, qualitative detection of Sapovirus RNA from human stool samples. The detection is done in a one-step real-time RT-PCR format where the reverse transcription is followed by the PCR in the same reaction tube. The isolated RNA is transcribed into cDNA by a reverse transcriptase. Gene fragments specific for Sapovirus (ORF1, if present) are subsequently amplified by real-time PCR. The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Sapovirus assay contains an Internal Control RNA (ICR) as an internal control of sample preparation procedure and/or to determine possible PCR-inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Enzyme Mix	1x	80 µl	red
R	Internal Control RNA	2x	1700 µl	brown
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Sapovirus multiplex real-time RT-PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platform	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
bioMérieux	NucliSENS easy®MAG™
Real-time PCR instruments	
Roche	LightCycler® 2.0, LightCycler® 480II, LightCycler® 480 z
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II and the LightCycler® 480 z
- RIDA®GENE Color Compensation Kit II (PG0002) for use with the LightCycler® 2.0
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (BioScience grade, nuclease-free)

7. Precautions for users

For *in-vitro* diagnostic use.
- This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed.
- The instruction manual for the test procedure has to be followed.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com

8. Collection and Storage

8.1 Sample preparation from stool samples

For RNA isolation of human stool samples, use a commercially available RNA extraction kit (e.g. RIDA® Xtract (R-Biopharm)) or RNA extraction system (e.g. Maxwell® RSC (Promega)). Extract viral RNA according to the manufacturer's instructions.
We recommend to dilute the stool sample before extraction 1:10 with water. Vortex intensely and centrifuge at 13,000 x g for 1 min. Use from the supernatant an appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Sapovirus assay contains an Internal Control RNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control RNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control RNA is used only as a PCR inhibition control, 1 µl of the Internal Control RNA should be added to the Master- Mix (s. Tab. 4).

If the Internal Control RNA is used as a extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control RNA has to be added during extraction procedure. The Internal Control RNA should always be

added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control RNA to the negative control and Positive Control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One Positive Control and one negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Enzyme Mix, the Positive Control, the No Template Control and the Internal Control RNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master Mix (ICR as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Enzyme Mix	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master Mix (ICR only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Enzyme Mix	0.7 µl	7.7 µl
R	Internal Control RNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the RT-PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control RNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control RNA to the RT-PCR-Mix of the negative control.

Samples: Add 5 µl RNA-Extract to the pre-pipetted Master-Mix.

Positive Control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control RNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control RNA to the RT-PCR Mix of the Positive Control .

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The RT-PCR reaction should be started according to the PCR instrument set-up (see Tab. 5, Tab. 6).

9.3 PCR instrument set-up

9.3.1 Universal real-time RT-PCR profile

Tab. 5: Universal real-time RT-PCR profile for LightCycler® series

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and extension occur in the same step.

Tab. 6: Universal real-time RT-PCR profile for Mx3005P, ABI7500, Rotor-Gene Q and CFX96™

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and extension occur in the same step

Note: The universal real-time PCR profile can also be used for DNA assays if RIDA®GENE DNA and RIDA®GENE RNA real-time PCR assays are combined in one run.

9.4 Detection channel set-up

Tab. 7: Selection of appropriate detection channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 2.0	Sapovirus	530	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	ICR	560	
Roche LightCycler® 480II	Sapovirus	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICR	533/580	
Roche LightCycler® 480 z	Sapovirus	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICR	540/580	
ABI 7500	Sapovirus	FAM	**Check that passive reference option ROX is none**
	ICR	VIC	
Qiagen Rotor-Gene Q	Sapovirus	Green	**The gain settings have to be set to 5**
	ICR	Yellow	
Agilent Techn. Mx3005P	Sapovirus	FAM	**Check that passive reference dye is none**
	ICR	HEX	
Bio-Rad CFX96™	Sapovirus	FAM	**-**
	ICR	VIC	

10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Positive Control and negative control have to show correct results (see Table 8, Fig. 1) in order to determine a valid run.

The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 8: For a valid run, the following conditions must be met:

Sample	Assay result	ICR Ct	Target Ct
Positive Control	Positive	NA *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

[1] No Ct value is required for the ICR to make a positive call for the Positive Control.

If the Positive Control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the Positive Control is valid prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

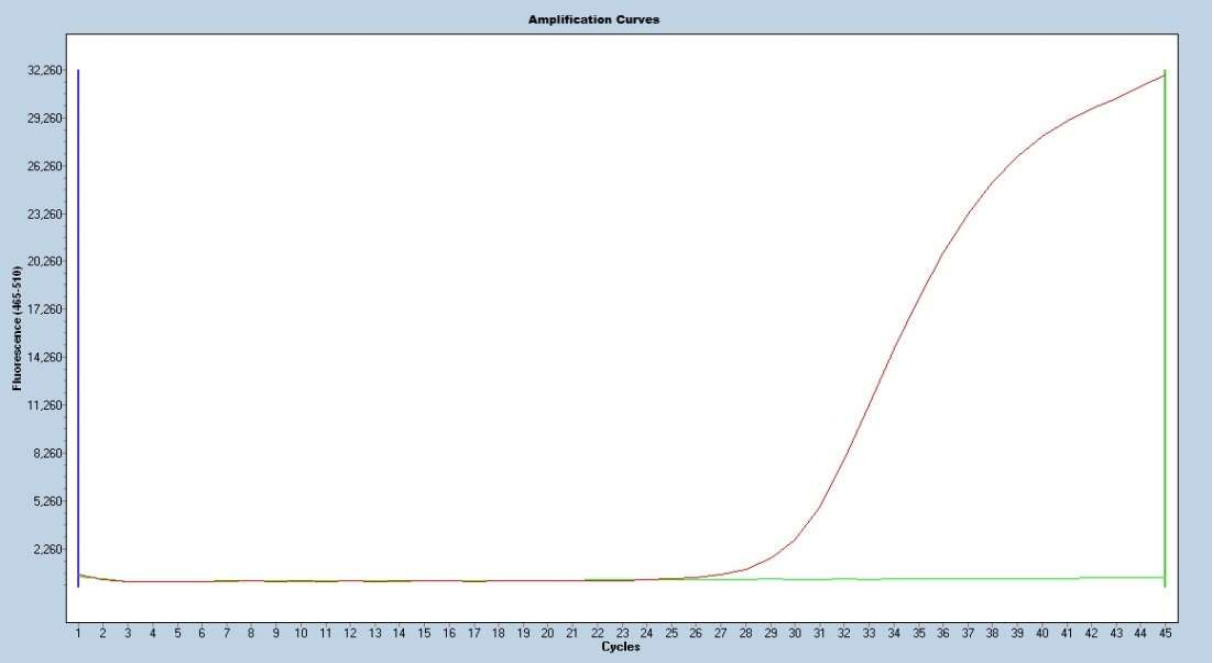

Fig.1: Correct run of the Positive Control and negative control (Sapovirus) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 9.

Tab.9: Sample interpretation

Sapovirus	ICR	Result
positive	**positive/negative**	**Sapovirus detected**
negative	**positive**	**Target gene not detected**
negative	negative	**Invalid**

Sapovirus is detected, if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

Sapovirus is also detected, if the sample RNA shows an amplification signal but none for the Internal Control RNA in the detection system. The detection of the Internal Control RNA is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control RNA.

Sapovirus is not detected, if the sample RNA shows no amplification signal, but an amplification signal for the Internal Control RNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control RNA.

A sample is invalid, if the sample RNA and Internal Control RNA show no amplification signal in the detection system. The sample contains a PCR inhibitor.

The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for stool samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Sapovirus assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target gene (ORF1).

13. Performance characteristics

13.1 Clinical performance

In a retrospective clinical validation study we analyzed 86 extracted human stool specimens with the RIDA®GENE Sapovirus assay and an in-house real-time PCR assay in an institute in France.

Tab.10: Correlation of the Sapovirus results with the RIDA®GENE Sapovirus multiplex real-time RT-PCR and reference in-house real-time PCR.

		In-house real-time PCR			
		Positive	Negative	Total	
RIDA®GENE *Sapovirus*	Positive	30	0	30	PPV: 100%
	Negative	0	56	56	NPV: 100%
	Total	30	56	86	

13.2 Analytical sensitivity

The RIDA®GENE Sapovirus multiplex real-time RT-PCR has a detection limit of ≥ 50 RNA copies per reaction for Sapovirus.

The following figure 2 shows a dilution series of Sapovirus (10^5 – 10^1 RNA copies per µl) on the LightCycler® 480II.

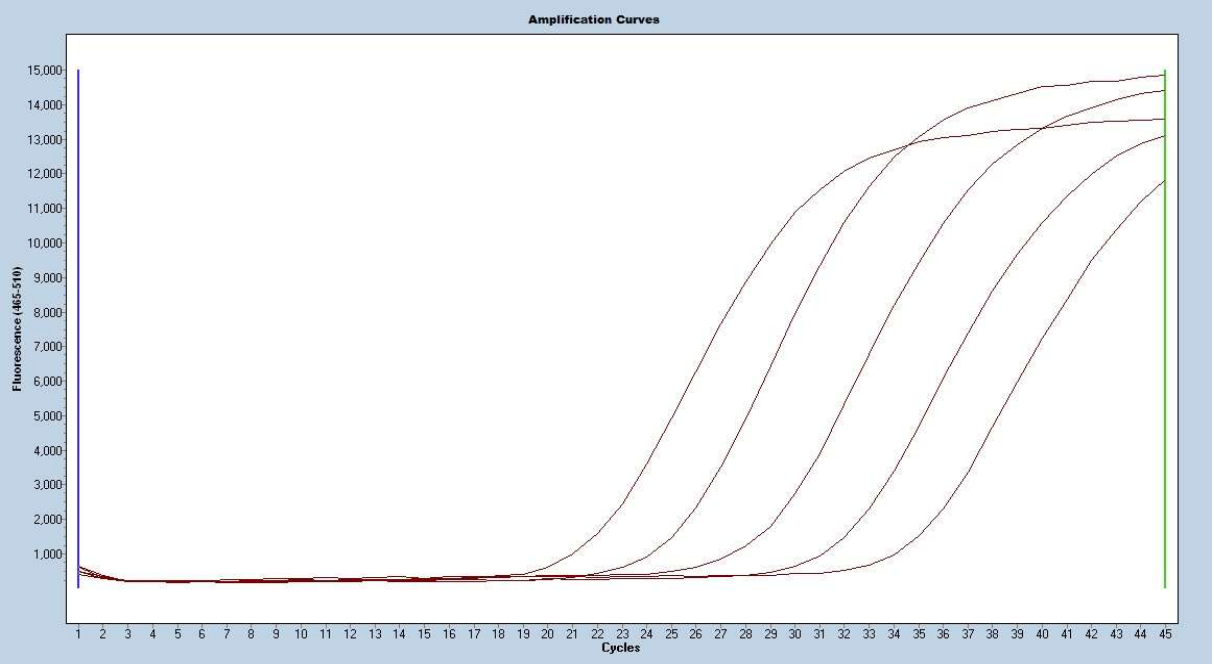

Fig. 2: Dilution series Sapovirus (10^5 – 10^1 RNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, RNA extraction and RNA concentration.

13.3 Analytical specificity

The analytical specificity of the RIDA®GENE Sapovirus multiplex real-time RT-PCR is specific for Sapovirus from human stool samples. No cross-reaction could be detected for the following species (see Tab. 11):

Tab. 11: Cross-reactivity testing

Adenovirus 1, human, strain Adenoid 71	-	*Campylobacter lari* subsp. *lari*	-	*Cryptosporidium parvum*	-	Norovirus GG II	-
Adenovirus 7, human, strain Gomen	-	*Campylobacter upsaliensis*	-	*E. coli* (O157:H7)	-	*Proteus vulgaris*	-
Adenovirus 40, human, strain Dugan	-	*Candida albicans*	-	*E. coli* (O26:H-)	-	*Pseudomonas aeruginosa*	-
Adenovirus 41, Human, Strain Tak	-	*Citrobacter freundii*	-	*E. coli* (O6)	-	Rotavirus	-
Aeromonas hydrophila	-	*Clostridium bifermentans*	-	*Entamoeba histolytica*	-	*Salmonella enteritidis*	-
Arcobacter butzleri	-	*Clostridium difficile*	-	*Enterobacter cloacae*	-	*Serratia liquefaciens*	-
Astrovirus	-	*Clostridium novyi*	-	*Enterococcus faecalis*	-	*Salmonella typhimurium*	-
Bacillus cereus	-	*Clostridium perfringens*	-	*Giardia lamblia*	-	*Shigella flexneri*	-
Bacteroides fragilis	-	*Clostridium septicum*	-	*Giardia intestinalis* Portland 1	-	*Staphylococcus aureus*	-
Campylobacter coli	-	*Clostridium sordellii*	-	*Giardia intestinalis* WB Clone C6	-	*Staphylococcus epidermidis*	-
Campylobacter fetus subsp. *fetus*	-	*Clostridium sporogenes*	-	*Klebsiella oxytoca*	-	*Vibrio parahaemolyticus*	-
Campylobacter jejuni	-	*Cryptosporidium muris*	-	Norovirus GG I	-	*Yersinia enterocolitica*	-

14. Version history

Version number	Chapter and designation
2018-06-11	Previous version
2021-02-01	General revision 10. Quality control (Spelling mistake) 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Reaction Mix

Enzyme-Mix

Internal Control RNA

No Template Control

Positive Control

16. Literature

1. Shigemoto N et al. Detection of *Norovirus*, *Sapovirus*, and human *Astrovirus* in fecal specimens using a multiplex reverse transcription-PCR with fluorescent dye-labeled primers. Microbiol Immunol. 2011, 55: 369-372.
2. Chiba S et al. Sapporo Virus: History and recent findings. J. Infect. Dis. 2000, 181(Suppl 2): S303-S308.
3. Johansson PJ et al. A nosocomial *Sapovirus*-associated outbreak of gastroenteritis in adults. Scand. J. Infect. Dis. 2005, 37: 200-204.
4. Lee LE et al. *Sapovirus* outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. Emerging Infect. Dis. 2012, 18: 873-876.
5. Nakata S et al. Prevalence of human *Calicivirus* infections in Kenya determined by enzyme immunoassays for three genogroups of the virus. J. Clin. Microbiol. 1998, 36: 3160-3163.
6. Wolfaardt M et al. Incidence of human *Calicivirus* and *Rotavirus* infection in patients with gastroenteritis in South Africa. J. Med. Virol. 1997, 51: 29